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A PINK YEAST CAUSING SPOILAGE IN OYSTERS.

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PURPOSE OF INVESTIGATION.

At certain periods during the last few years the oyster growers of New England have had difficulty in shipping oysters for long distances without the development of a pink color in the liquor or on the oyster itself. Analyses of shipments showing this color have demonstrated the cause. The organism producing the pink pigment is a yeast-like fungus which grows readily at low temperature, and produces pigment bright enough to be noticed only when the shipment has been in transit three or four days. Oysters which appear to be in good condition when shipped are often found to be pink when they reach their destination.

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[1] The major portion of this investigation was carried on at Brown University, Providence, R. I., under the supervision of Prof. F. P. Gorham, and was submitted to the faculty of that institution in partial fulfillment of the requirements for the degree of doctor of philosophy. During the early part of the investigation valuable assistance was given the writer by Charles W. Parmelee. The work was completed and the report revised in the Microbiological Laboratory of the Bureau of Chemistry. Valuable criticisms and suggestions have been offered by Dr. Charles Thom of that laboratory.

The investigation here reported was undertaken to determine the distribution of this "pink yeast," and to find a suitable method of controlling or preventing its development. Later, the work was extended to cover a more detailed study of the organism itself, including its morphology, physiology, and biochemistry.

While much concerning the pink yeast and its relation to the oyster industry remains to be studied, the data given in this bulletin may help to a better understanding of the cause of the color, the distribution of the organism, its control, and some of its physical and chemical properties.

HISTORICAL REVIEW.

The true yeasts, Saccharomycetes, and the pseudo yeasts, or Torulaceæ, are widely distributed, and have been isolated from many sources. L. van der Hulle and H. van Laer (1890) isolated a pink yeast from Belgian beer. M. Ward (1892) studied a pink yeast which was an alien organism in ginger beer yeast. Kramer (1891) obtained a pink yeast-like form from must fermentation, while V. Peglion (1895) and E. Kayser (1890) also studied pink yeasts from must fermentation. R. Demme (1890) studied and described a pink form from milk and cheese, and pink yeasts from milk and butter have also been described by Krueger (1892) and R. Reinmann (1900). B. Fischer and K. Brebeck (1894) describe pink yeasts from the stomach of a patient suffering from gastric fermentation, also a pink yeast from samples of sea water south of the Azore Islands.

The opinion that Torulaceæ are merely stages in the development of other fungi has been expressed by many workers, notably by Hansen (1898). E. Klein and N. Gordan (1912) claim that a certain pink yeast originates in a rust infecting the Canada thistle, *Puccinia suaveolens*. On the other hand, R. Meissner (1899) has shown differences between the budding Torula forms and the budding cells of the fungus causing peach leaf curl, *Exoascus deformans*. It is claimed by Laurent (1889) that the budding spores of *Cladosporium herbarium* become pink yeasts. The earliest attempt to study these pink or red yeasts was made by F. A. Janssens and A. Mertens (1903) with an organism described as a "red Torula." Such forms, which have been known for a long time, were first described by Fresenius (1850) as *Cryptococcus glutinis*. Later Schröter and Cohn (1872) grouped the pink yeast with the Saccharomycetes, but Hansen has shown that *Cryptococcus glutinis* comprises several species and can not be included in the Saccharomycetes. Hansen (1902) and P. Lindner (1887) were unable to see any sporulation in the red budding fungi, and Elfving (1886) also studied a pink yeast which did not form spores. A. P. Swan (1896) described a red budding fungus in which he claims to have observed sporulation, but J. Chr. Bay (1896) disputed this in a subsequent publication.

Although frequent mention has been made in various trade journals of the occurrence of the pink color in oysters, no detailed study of the relation of the pink yeast to the oyster industry has been published thus far. Bates and Round have submitted to the Bureau of Chemistry a preliminary report on the occurrence of a pink yeast in oysters during the fall of 1914. Based upon their report, a statement was made in the Report of the Chemist[1] that "It was discovered that a yeast was the probable cause of the reddening which affected thousands of gallons of oysters during the past season."

From the foregoing paragraphs it is evident that these pink yeasts are widely distributed in nature. Owing to a lack of adequate description of these forms, no attempt has been made to identify the organism reported here with any of the forms mentioned other than that they all belong to the same large group of Torulæ.

DISTRIBUTION OF THE PINK YEAST.

After the cause of the color had been found to be a pink yeast the next thing undertaken was a study of the distribution of the organism in the oyster house, in oysters, in surface and deep water samples from Narragansett Bay, and in mud samples taken from the oyster beds. If the distribution was limited to the house, the solution of the problem was simple, consisting merely of finding some suitable method of removing the infection from everything with which the oysters came in contact. If, on the other hand, the habitat of the yeast extended to the water and mud on the oyster grounds, the problem became more complex, with less prospect of eliminating the trouble. An examination was first made of the oyster house and of the utensils in and about the house. Later oysters taken from the decks of the boats were examined before they had entered the house, and finally numerous samples of water and mud were analyzed for the presence of the yeast.

Culture media.—After several preliminary experiments with special culture media of various kinds it was decided that satisfactory results would be obtained with dextrose agar and dextrose broth. These media, upon which the yeast grows abundantly, have been used throughout the work. The agar and broth were prepared according to the directions given in the Standard Methods of Water Analysis of the American Public Health Association. Beef extract, peptone, and pure dextrose were used, and the reaction was adjusted to +0.5 Fuller's scale. One per cent of dextrose has been found sufficient.

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[1] U. S. Dept. Agr. An. Repts., 1915.

IN AND ABOUT THE OYSTER HOUSE.

METHOD OF EXAMINATION.

Large swabs of cotton on wooden rods were made and sterilized and kept in large jars plugged with cotton. A swab was dipped in dextrose broth until thoroughly wet, and then the moistened swab was rubbed lightly over the area under examination. The swab was then returned to the tube of dextrose broth, marked with the necessary data, and returned to the laboratory. Immediately on arriving at the laboratory the dextrose broth tubes in which the swabs were placed were incubated at room temperature (21° to 25° C.) for 12 to 24 hours, after which the broth was plated out in the proper dilutions on dextrose agar. The plates were incubated at room temperature for 7 days, being examined daily for the presence of pink colonies. Whenever a pink colony was found it was taken from the plate with a sterile needle and transferred to an agar slant. At the same time a smear was made for microscopic examination. The colonies are very characteristic, both in color and in general appearance, and can be recognized without difficulty. A smear made from each suspected colony was examined, however, in order to avoid error in recording results.

RESULTS.

In the tables which follow letters have been substituted for the names of boats, while the areas from which samples were taken are designated by numbers.

TABLE 1.—*Results of examination of house and utensils.*

Date.	Location examined.	Result.
1916.		
Sept. 7	Sides and floor of second bin, south side	0
Do.	Dust from floor of fourth bin, south side	+
Do.	Sides and floor of fourth bin, south side	+
Do.	Opening bench in front of bins, south side	+
Do.	Opening bench in front of bins, south side (men working)	0
Do.	Opening bench in front of bins, north side	0
Do.	Shells from bench, south side, that have rubbed around the bench	+
Do.	Oysters from the pile in the bin (outside of shell examined)	+
Do.	Shucked oysters as passed into washroom	0
Sept. 18	Deck of the steamer A	+
Do.	Deck of the steamer A (men working at the time)	+
Do.	Oysters in shell from deck of the boat (oyster meats examined)	0
Do.	Inside the tub on steamer A	+
Do.	Inside of wheelbarrow 9	+
Do.	Inside of wheelbarrow 3	+
Do.	Oysters in shell from deck of steamer E (oyster meats examined)	0
1917.		
Sept. 4	Sides and floor of fourth bin, south side	+
Do.	Sides and floor of seventh bin, south side	+
Do.	Opening bench, south side (new boards)	+
Do.	Opening bench, north side (men working)	+
Do.	Sides and floor of fourth bin, north side	+
Do.	Sides and floor of seventh bin, north side	+
Do.	Window sills on the south side	+

Table 1 shows that the pink yeast is widely distributed throughout the oyster house and the adjacent structures, and can be found on the decks of the boats, 73.9 per cent of the locations examined giving positive results. At the oyster house where the investigation was made the oysters are hoisted from the decks of the boats in tubs, then dumped into deep wooden wheelbarrows and carried back along the runway to be emptied into the bins within the house. Starting at the bins and the opening benches, the pink yeast was found to be rather abundantly distributed on the sides and floors of the bins. The bins first examined in 1916 had not been used since the preceding April, so that the dust from several summer months had accumulated in them. This dust contained large numbers of the yeasts. Attention was next directed to the wheelbarrows in which the oysters are carried, swabbings from the inside of two of which showed that the pink yeasts are to be found there also. Swabbings made from the tubs in which the oysters are hoisted also showed the presence of the yeasts. Going still further back in the search, an examination of the swabbings made of the decks of the boats showed pink yeasts. Oysters from the decks examined at this time showed no yeasts. In examining the oysters from the decks of the boats, the oyster meats within the shell were analyzed, but the outsides of the oyster shells were examined in the sample taken from the bin within the house. These results show that the oyster house itself and the utensils on the premises are, at least, sources of the infection.

IN OYSTERS.

To ascertain whether or not the oysters already contained the yeasts when taken from the waters of the bay, further analyses were made.

Method of Analysis.

Visits to the docks were made at the time when the steamers were to arrive, and samples were obtained before the work of unloading had begun. A representative sample of 15 or 20 oysters was taken from the middle of the pile on the deck, and transported to the laboratory in a cardboard box. The oysters were scrubbed thoroughly with a stiff brush in warm water and soap. They were then opened aseptically, and 3 oysters placed in a sterile jar with their liquor. In opening the oysters the knife was sterilized with flaming alcohol, and the edge of the oyster shell was passed through the flame before opening. The opened oysters in the jar were vigorously shaken 50 times and dilutions made of the liquor. The proper dilutions[1] were plated on dextrose agar and examined as already described.

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[1] 0.1 cc. and 0.01 cc. were found to be adequate.

Later in the course of the work trips were made to the oyster beds, where the samples were taken just as the oysters were brought out of the water, thus avoiding the contact of the oyster with the deck, which was known to be infected. Such samples, placed in cardboard boxes, were kept in a cool place until they could be taken to the laboratory and analyzed. This was usually several hours later, but, inasmuch as the analysis was qualitative rather than quantitative, the delay has not affected the results.

TABLE 2.—*Analyses of oysters from the bay.*

Date.	Source.	Result.
1916.	Steamer:	
Nov. 12	B	0
Dec. 4	B	0
1917.		
Jan. 8	A	0
Jan. 30	A	1 colony.
Feb. 5	A	0
Feb. 21	E	0
Mar. 1	B	0
Mar. 12	B	1 colony.
Mar. 15	B	0
Mar. 19	C	1 colony.
Mar. 22	A	0
Mar. 26	Schooner from Long Island	2 colonies.
Mar. 29	Steamer D	Do.
Apr. 4	Dredge on A, as taken from water	0
Apr. 12	do	0
Apr. 26	do	0
May 24	Dredge on C, as taken from water	0
May 29	Seed oysters taken back from the shop during winter. Taken from bed 4	0
May 31	Old oysters taken at bed 6	0
June 4	do	0
June 8	do	2 colonies.
June 12	do	Do.
June 14	Oysters taken at bed 2	0
June 21	Old oysters taken at bed 6	1 colony.
June 26	Oysters taken at bed 4	2 colonies.
June 28	do	1 colony.
July 3	Deck of Steamer B	0
July 5	Old oysters taken at bed 5	0
July 9	Oysters taken at bed 2	0
July 11	do	0
July 16	Oysters taken at bed 6	0
July 19	Oysters taken at bed 3	0
July 24	Young oysters clinging to starfish mops at bed 2	0
July 31	do	0
Sept. 13	Oysters taken from bed 4	0
Do.	Oysters taken at bed 6	0

RESULTS.

The results recorded in Table 2 show that the pink yeasts may also be found in oysters before they have been handled or brought in contact with infected objects. The number of positive results was not as great in the case of the oyster analyses as in the case of analyses made from the oyster house, only 10 oyster samples of the 38 examined, or 26.3 per cent, showing the presence of the yeasts. There was also a decided difference in the number of colonies to be found on the plates made from samples from the two sources.

In no case were there more than two colonies of pink yeasts on one plate in the oyster samples, while the plates made from swabbings from the oyster house frequently presented a surface actually dotted with the pink colonies. An attempt to determine whether or not the infection was localized and whether oysters coming from particular localities were infected failed. As the table shows, yeasts were recovered from oysters taken from beds 6 and 4, two areas which are widely separated, bed 4 being east of Prudence Island and bed 6 some distance west of the island. No relation between the season of the year and the presence of the yeasts could be found, positive results being obtained in midwinter as well as in midsummer. It is interesting to note that oyster samples taken from the deck of a schooner from Long Island on March 26 before these oysters had ever been in the waters of Narragansett Bay showed the presence of yeasts. It may be concluded from Table 2 that the oysters are infected before they reach the city, but a comparison of the results in Table 2 with those in Table 1 shows that the chance of infection is far greater in the house than down the bay.

IN WATER.

To determine whether or not the pink yeasts were to be found floating in the water of the bay or whether they were in the mud and soil on the bottom, analyses of surface and deep water samples have been made.

Method of Obtaining and Analyzing Samples.

The surface water samples were collected in sterilized 14-ounce French tincture bottles in the usual manner. After the sample was collected, the bottle was labeled and set in a cool place, to be carried later to the laboratory. The samples were then plated according to the Standard Methods of Water Analysis on dextrose agar, and the plates incubated and examined as described on page 4.

For collecting the deep water samples, which has presented more difficulties, a special apparatus, devised and used by the Rhode Island Shellfish Commission in its investigation of the waters of Narragansett Bay, was employed. It consists of a heavy piece of brass, 20 cm. long by 4 cm. wide and 2 cm. thick. About 2 cm. from the top of the piece of brass is a slit. A knife edge fitted to slide over the back of the apparatus will pass over the opening of the slit. On the front of the brass piece are two clasps for holding a special tube made from an ordinary test tube, which is heated in the flame and drawn out to a capillary point at right angles to the body of the tube; the whole tube is then heated to drive out the air and sealed, forming a partial vacuum tube. The body of the tube is held in the clasps on the apparatus, and the capillary point passes through the slit and extends out in back. When ready to take a sample of deep water, the tube is placed in the clasps as described, and the apparatus, suspended from a strong cord, is lowered to the required depth. A heavy lead weight, which is pierced so that it will slide over the cord, is held in the hand until the apparatus is at the necessary depth. The weight is then allowed to slide down the cord. When it reaches the apparatus it strikes the sliding knife edge hard enough to cause the edge to slide down, breaking the capillary point of the tube. The tube will then fill up with water to about two-thirds of its capacity, and may be lifted up without any further water being taken in. In this manner a sample of from 15 to 25 cc. can be secured from any depth. Samples thus obtained were carried in a specially constructed rack to the laboratory. The tops of the tubes were then broken, precautions being taken to avoid contamination, and the samples were analyzed in the same manner as surface water samples.

TABLE 3.—*Analyses of surface and deep water samples.*

Date.	Bed No.	Samples.		Result.		Date.	Bed No.	Samples.		Result.	
		Location.	Number.	Positive.	Negative.			Location.	Number.	Positive.	Negative.
1917.						1917.					
Mar. 26	6	Surface	4	0	4	June 14	2	Surface	3	0	3
		Deep	9	0	9			Deep	8	1	7
Apr. 4	6	Surface	3	0	3	June 21	6	Surface	3	0	3
		Deep	12	0	12			Deep	8	0	8
Apr. 12	6	Surface	3	0	3	June 26	4	Surface	3	0	3
		Deep	12	0	12			Deep	8	0	8
Apr. 26	7	Surface	2	0	2	June 28	4	Surface	3	0	3
		Deep	10	0	10			Deep	8	0	8
Do.	5	Surface	1	0	1	July 3	1	Surface	3	0	3
		Deep	2	0	2			Deep	7	0	7
May 22	4	Surface	3	0	3	July 5	5	Surface	3	1	2
		Deep	11	0	11			Deep	8	3	5
May 24	6	Surface	3	0	3	July 9	2	Surface	3	0	3
		Deep	8	0	8			Deep	8	0	8
May 29	3	Surface	3	1	2	July 11	2	Surface	2	0	2
		Deep	8	1	7			Deep	8	0	8
May 31	6	Surface	3	0	3	July 16	6	Surface	4	0	4
		Deep	6	2	4			Deep	8	0	8
June 4	6	Surface	3	0	3	July 19	3	Surface	4	0	4
		Deep	8	0	8			Deep	7	0	7
June 8	6	Surface	3	0	3	July 24	2	Surface	4	0	4
		Deep	8	0	8			Deep	8	0	8
June 12	6	Surface	2	0	2	July 21	2	Surface	3	0	3
		Deep	6	0	6			Deep	8	0	8

RESULTS.

The analyses of water samples give a much smaller number of positive results than do analyses of the oysters or swabbings from the oyster house. But 2 positive samples of surface water have been found in the 71 samples examined, or 2.8 per cent, and but 7 positive samples of deep water in the 194 examined, or 3.6 per cent. The great difference in the number of positive samples obtained from the various sources is apparent when the percentages are compared, 73.9 per cent in the house, 26.3 per cent in oysters, 3.6 per cent in bottom samples, and 2.8 per cent in surface samples. In all the positive cases the number of colonies on the plates was very small in comparison with the number found when examining the oyster house. The water of Narragansett Bay may be considered almost a negligible factor in the contamination of the oysters with the pink yeast. Beds 1 and 7 are widely separated, and extend from above Conimicut Point to the lower part of the bay. Hence in Table 3 it may be noted, as in Table 2, that the positive samples were obtained from areas a great distance apart, and no conclusions can be drawn as to the distribution of the organism in particular localities.

A record of the weather conditions and the temperature of the water has been kept, in the hope that some relation might be found between such conditions and the presence of the yeast, but the very small number of positive results obtained and the fact that these positive results were obtained from localities distant from each other has made such data of no value. It may be said, however, that, as far as can be deduced from the data at hand, there is apparently no relation between the tide, wind, and current and the distribution of the pink yeast in the water.

IN MUD.

Method of Collecting and Analyzing Samples.

Whenever the bottom was soft and muddy it was a simple matter to obtain the sample. A large dredge was dragged along over such bottom, and then hoisted to the side of the steamer, while a small amount of the material was taken from the dredge by means of a sterile spoon and transferred to a sterile glass jar. When the bottom was hard and sandy, a greater length of chain was let out on the dredge and the speed of the boat was slowed down as much as possible. If the dredge was left down long enough there was no difficulty in securing enough of the sand for analysis. The glass jars containing the samples were placed in the refrigerator on the boat until they could be taken to the laboratory. Samples of mud and sand were analyzed according to the usual methods of soil analysis. A small amount of the material, taken from the jar with a sterile spatula, was thoroughly shaken with 10 cc. of sterile water, and dilutions of this mixture were made. Since the examination was not quantitative, no attempt was made to weigh out or measure known amounts of the muddy material. The plate cultures were made as before described, and examined daily.

RESULTS.

In no case was the pink yeast recovered from the 20 samples of mud and sand collected. It may be concluded that the soil on the bottom of the bay is not a factor in the contamination of the oysters with the pink yeast.

A discussion of the distribution of the yeast and its relation to the oyster industry, together with recommendations for its control, is given on page 21.

EXPERIMENTAL PRODUCTION OF PINK COLOR IN OYSTERS.

In order to prove conclusively that the pink yeast is the cause of the pink color in shipments of shucked oysters, several experiments were undertaken to produce the color by the introduction of cultures of the organism into jars of normal oysters.

Two gallons of oysters, obtained in the market, were divided among 9 glass jars. Five cubic centimeter amounts of a pink yeast culture, grown in an oyster broth medium for 4 months, were introduced into 6 of the jars. The remaining 3 jars were held as controls. Two inoculated jars and a control jar were placed in the cold storage at 0.5° C., in the refrigerator at from 8 to 10° C., and directly on the ice at about 5° C. These jars were examined daily, the presence or absence of a pink color noted, and the temperature within the jars recorded. At the end of the first week the oysters in the jars on the ice and in the refrigerator were decidedly pink. There was no pink color in the jars of oysters used as controls. At the end of one month the oysters in the cold storage at 0.5° C. were slightly pink, while the control oysters were not pink. The pink yeast was recovered from each jar which showed a pink color. In each jar, however, the oysters were spoiled and very sour by the time they were pink. The oysters used for the experiment were shucked with their own liquor, and were delivered to the laboratory without being washed. They contained a large amount of dirt and many bacteria in the mucus which, without doubt, brought about the rapid spoilage.

In order to produce the pink color in oysters properly washed and shipped the following experiment was carried out. A trip was made to a near-by city and 3 gallons of oysters purchased at the packing house. These oysters had been carefully shucked and washed in a blower, and then placed in new, clean 1-gallon cans. Into each of two 1-gallon cans of oysters were introduced about 25 cc. of a 10-day oyster broth culture of the pink yeast. The third can was left as a control. The cans were closed and packed in a wooden box with cracked ice, and shipped to the laboratory just as they would be shipped to the retailer. These oysters were 5 days in reaching the laboratory, and when they were received they were light pink in color. The control can was normal. All 3 cans were placed in cold storage, and in 2 days the 2 cans were decidedly pink, the control remaining normal. In this experiment also the pink yeast was recovered from the pink oyster liquid.

These experiments, together with the fact that the pink yeast was isolated from every shipment of pink oysters examined, are good evidence that the pink yeast is, without question, the cause of the pink color in shucked oysters. The experiments have also shown that when oysters are pink they are close to the stage of spoiling, as it takes 5 or 6 days for the color to develop at a temperature of about 10° C. When kept at a temperature as low as 0.5° C. about 1 month is required to produce the pink color, in which time the oysters become decidedly stale.

MORPHOLOGY OF THE PINK YEAST.

Shape and size.—Elliptical. Maximum measures 9.8 by 7 micra; minimum measures 4.1 by 2.8 micra. Average measurement, 6 by 4 micra. No mycelium. Often grouped in gelatinous framework. Granules, fat globules, and vacuoles present. Budding forms common.

Sporulation.—Yeast grown for 1 month on sterilized gypsum blocks and examined at intervals showed no spores, either when unstained or when stained with carbol fuchsin.

Staining reactions.—Stains readily with methylene blue, safranin, or gentian violet. Not acid fast. Staining by Gram's method is very similar to that reported by Henrici (1914). Decolorized with 95 per cent alcohol, the cells are Gram positive, but a few cells in the field are partly decolorized, showing a number of fine black particles in a red matrix. When 30 per cent acid (HCl) alcohol is used the cells are completely decolorized.

CULTURAL FEATURES OF THE PINK YEAST.

Temperature.—Optimum temperature, 22° C. Will not grow at 37° C. Grows at 6° C., but growth not as abundant nor as characteristic as at 22° C.

Dextrose broth.—Growth first appears as precipitate in bottom of tube. Later a ring forms around wall of tube at surface of medium. Precipitate flocculent. No film formation.

Dextrose agar slant.—Growth along the streak, heavy, moist, and glistening. Later growth collects at base of slant in heavy mass. Growth of slimy consistency.

Gelatin stab.—Growth fair. Uniform along the stab. Pigment production not pronounced. No liquefaction.

Dextrose agar colonies.—Visible about third or fourth day. Round, smooth, moist, and glistening.

Litmus milk.—Same as broth cultures. No coagulation nor peptonization.

Blood serum.—Growth moderate. Similar to agar slant. No liquefaction.

Potato.—Growth moderate. Similar to agar slant. No destruction of starch.

BIOCHEMICAL FEATURES OF THE PINK YEAST.

ACID PRODUCTION.

To determine whether or not acid was produced by the pink organism, cultures were made in various carbohydrate media, and titrations made daily. Dextrose, maltose, lactose, raffinose, saccharose, and glycogen broths were used. Of these the first four were prepared according to the standard methods of the American Public Health Association.

Inasmuch as the pink yeast was found growing in and was isolated from oyster liquor which contains a large amount of glycogen, it was thought necessary to test its acid production in this carbohydrate. Owing to the scarcity of glycogen in pure form and to the difficulty in preparing it, a special medium containing glycogen was made. An infusion of oysters was prepared by infusing finely ground oysters over night with double the quantity of water. The mixture was allowed to boil for 2 minutes over the flame, after which it was strained through a cloth. To the resulting oyster infusion 1 per cent peptone was added, and the broth made neutral. As oysters contain glycogen in amounts varying from 3 per cent to 25 per cent of the total solids according to the season, food supply, etc., the broth prepared no doubt contained a fairly high percentage of glycogen. Growth in this oyster broth at room temperature resembles the conditions under which the yeast occurs, and it is of special interest to test for acid production in this medium. All these media were titrated after sterilization and before inoculation. Thereafter titrations of the cultures and controls were made daily for 7 days. The titrations were made against N/20 sodium hydroxid, using phenolphthalein as an indicator.

In addition, acid production was determined in cultures grown anaerobically, in order to approximate the conditions afforded for the growth of the pink yeast in oysters packed in sealed glass jars. The cultures were grown under paraffin oil and titrated daily as described.

In none of the carbohydrate media used was there any acid production by the pink yeast when grown either aerobically or anaerobically.

ALCOHOL, ALDEHYDE, AND ACETONE PRODUCTION.

The production of alcohol in sugar solutions is without doubt the best known of all properties of the yeast plant. Alcoholic fermentations due to the growth of fungi have been known for a long time, and it was because of the alcoholic fermentation of sugars that the true yeasts were given the name Saccharomycetes. One of the distinguishing features of the Torulæ or pseudo yeasts, however, is the fact that they cause alcoholic fermentation to a very slight degree or not at all. In studying the pink yeast, experiments were conducted to determine whether or not this organism produces alcohol in various sugar media.

The pink yeast was grown at room temperature in dextrose, levulose, maltose, lactose, galactose, raffinose, arabinose, saccharose, glycogen, inulin, and dextrin broths for 72 hours with controls. At the end of the incubation period the cultures and controls were attached to a condenser and distilled. The distillate was tested for alcohol, aldehyde, and acetone by routine methods, and no positive results were obtained. Applied to a culture of a known alcohol-producing yeast these methods gave positive tests. The pink yeast does not give the test for alcohol, aldehyde, or acetone when grown under the conditions described, but whether small amounts of these compounds may be formed under other conditions was not determined.

GAS PRODUCTION.

Experiments were also conducted to determine the gas production of the pink yeast. Dextrose, maltose, lactose, raffinose, saccharose, and glycogen broths were prepared as described. Fifteen cubic centimeters of each medium were used in Dunham fermentation tubes. After inoculating with the pink yeast the tubes were incubated, with controls, at room temperature, and for 7 days examined for the production of gas. Cultures were also grown anaerobically under paraffin oil.

In none of the tubes was any gas produced in 7 days. The tubes were reserved for the examination at a later period, and no gas production was observed for 21 days.

ENZYME PRODUCTION.

Enzyme production is a property possessed by many yeasts. Most of the true yeasts produce invertase, which inverts saccharose to dextrose and levulose. Certain yeasts also produce protease and diastase. Experiments were conducted to determine whether or not protease, diastase, invertase, or rennin are produced by the pink yeast.

Protease.—Fifty cubic centimeters of blood serum and milk serum, filtered through a porcelain candle, were prepared, inoculated, and incubated at room temperature for 3 days. For control purposes cultures of *B. proteus*, which is known to produce a proteolytic enzyme, were also made, and flasks of serum which had not been inoculated were likewise incubated with test cultures for controls. At the end of the incubation period the liquid was made faintly acid with acetic acid, and boiled to precipitate the unaltered proteins. These were filtered out, and the filtrate was tested for polypeptids, peptones, tyrosine, and tryptophane with copper sulphate and sodium hydroxid, Millon's reagent and glyoxylic acid.

Diastase.—Tube cultures of the pink yeast were prepared in sugar-free broth. As controls, cultures of *B. subtilis*, which produces diastase, and sterile broth tubes were incubated for controls, and all the cultures were incubated at room temperature for 3 days. A thin starch paste with 2 per cent thymol was prepared, and equal parts of the cultures and the paste were mixed. The mixtures were incubated for 6 hours at 37° C., and then filtered. The filtrates were tested for sugar with Fehling's solution.

Invertase.—In testing for the production of invertase, tube cultures were made of the pink yeast in sugar-free broth as in testing for diastase. Cultures were also made of the *Saccharomyces cerevisiæ*, which inverts saccharose, and uninoculated cultures were incubated for controls. A 2 per cent saccharose solution was made, and 2 per cent phenol added. Equal parts of the cane-sugar solution and the cultures were mixed and allowed to stand for several hours. They were then filtered, and the filtrates tested for reducing sugar with Fehling's solution.

Rennin.—Tube cultures of the pink yeast and of *B. prodigiosus* were prepared in sugar-free broth, and incubated for 3 days with uninoculated cultures for controls. At the end of the incubation period the cultures were heated to a sufficient temperature to sterilize. By means of a sterile pipette 5 cc. of the culture were introduced into each of 3 litmus milk tubes. The litmus milk tubes were placed in the incubator at 22° C., and examined daily for 10 days.

TABLE 4.—*Enzyme production.*

Enzyme.	Organism.	Result.	Enzyme.	Organism.	Result.
Protease	Pink yeast	0	Invertase	Pink yeast	+
	B. proteus	+		*Sacch. cerevisiæ*	+
	Control	0		Control	0
Diastase	Pink yeast	0	Rennin	Pink yeast	0
	B. subtilis	+		*B. prodigiosus*	+
	Control	0		Control	0

The results in the table indicate that no proteolytic, diastatic, nor milk-coagulating enzyme is formed by the pink yeast, but that the yeast does produce invertase which changes saccharose to dextrose and levulose.

INDOLE PRODUCTION.

In order to test for the production of indole by the pink yeast, cultures were grown for 15 days in tryptophane broth at room temperature, with controls, and were tested daily. The tests were made with paradimethylaminobenzaldehyde and hydrochloric acid. No positive tests were obtained.

AMMONIA PRODUCTION.

The power of the pink yeast to reduce nitrates to nitrites and ammonia was tested. The yeast was grown in peptone-nitrate solution for 10 days, and from the fifth to the tenth day the cultures and controls were tested for the presence of nitrites and ammonia. Sulphanilic acid and α-naphthylamin were used in testing for nitrates, while Nessler's reagent was added to the cultures in testing for ammonia.

Table 5.—*Production of nitrites and ammonia.*

Organism.	Result.									
	6 days.		7 days.		8 days.		9 days.		10 days.	
	Nitrites.	Ammonia.	Nitrites.	Ammonia.	Nitrites.	Ammonia.	Nitrites.	Ammonia.	Nitrites.	Ammonia.
Pink yeast	+	+	+	+	+	+	+	+	+	+
Control	0	0	0	0	0	0	0	0	0	0

The results of the experiments show that the nitrates in the culture medium are reduced to nitrites and ammonia.

PHENOL PRODUCTION.

Cultures were prepared in nutrient broth in flasks containing at least 50 cc. of medium, and were incubated at room temperature for 3 days with controls. At the end of the 3 days 5 cc. of 25 per cent sulphuric acid were added to the culture, and the mixture was distilled. The distillate was tested with Millon's reagent, ferric chlorid solution, and bromin water. Both the test and control cultures gave no indication of phenol.

PIGMENT PRODUCTION.

When grown under favorable conditions, the yeast produces abundant pink pigment. The most abundant pigment production is obtained when the yeast is grown upon solid sugar media at room temperature. The pigment is also produced in liquid media, but in much smaller quantity than on solid media. When grown at refrigerator temperature no pigment is produced until the fourth or fifth day, and then only a very slight amount. The pigment is not red or rose-colored, as the name "red Torula" or "red yeast" often given to the organism by investigators would indicate, but is more nearly the color of the ordinary pink coral. Its color matches the Orange Red Tint No. 1 of the Bradley educational colored papers. Chapman (1916), who studied the coloring matter of "red Torulae," has found it to be a substance similar to carotin. In the present investigation of the pigment of the yeast an attempt has been made to determine first a method of dissolving it, and, second, its solubility in various substances. After several experiments with different methods the following manner of dissolving the pigment has been found most productive of results. The yeast was grown on large agar slants for 3 days at room temperature, at the end of which time the growth was transferred to a small mortar and thoroughly ground with fine sand to break the yeast cells. After grinding with the sand the mixture was kept at about 40° C. until completely dried, and then ground again with the solvent to be tested. The mixture was filtered, and if the pigment was soluble the filtrate obtained was of a clear pink color.

Using this method, it has been found that the coloring matter is insoluble in hot and cold water, is slightly soluble in alcohol and carbon disulphid, somewhat more soluble in chloroform, and very soluble in ether. As such solutions of pigment slowly lose their color upon standing in the light, at the end of 2 weeks the chloroform and alcohol solutions were colorless. Ether solutions left standing in the light for 1 month are completely decolorized.

PHYSICAL FEATURES OF THE PINK YEAST.

RELATION TO OXYGEN.

Five methods were used in studying the growth of the pink yeast under anaerobic conditions.

Method 1 (*Hesse's method*).—Stab cultures were made in dextrose agar, choosing for the purpose a straight tube containing a deep column of medium and thrusting the inoculating needle to the bottom of the tube. A layer of sterile paraffin oil, 1 to 2 cm. deep, was poured on the surface of the medium, and the culture was incubated at room temperature with a control tube of agar which had not been inoculated.

Method 2.—A tube of agar was liquefied by heat, poured into a sterile Petri dish, and allowed to solidify. The surface of the medium was inoculated with the pink yeast in one spot only. A cover slip was taken from a jar of alcohol with sterile forceps, and the alcohol burned off in the flame. The sterile cover slip was lowered on the medium over the inoculated spot, carefully excluding air bubbles, and was then pressed down firmly with the points of the forceps. This plate was incubated at room temperature with a control plate.

Method 3 (*Roux's physical method*).—Tube cultures of liquid media were prepared as usual, and some of the inoculated media was aspirated into capillary pipettes. The ends of the capillary pipettes were carefully sealed in the flame, and the pipettes with their controls were incubated at room temperature.

Method 4 (*Buchner's method*).—Tube cultures were prepared as already described. Ten cubic centimeters of "acid pyro" solution were placed in a large Buchner's tube, and to this was added 1 cc. of N/10 sodium hydroxid, or 2 cc. of N/20 sodium hydroxid. The inoculated tube was placed inside the Buchner tube, and the mouth of the tube fitted tightly with a moistened rubber stopper. The tubes were incubated with controls.

Method 5 (*Wright's method*).—After tube cultures had been prepared as usual, the portion of the cotton-wool plug projecting above the mouth of the tube was cut off with scissors, and the plug pushed into the tube for a distance of 2 or 3 cm. By means of a pipette about 1 cc. of 10 per cent aqueous solution of pyrogallic acid was dropped onto the plug. It was absorbed immediately. By means of another pipette, an equal amount of N/10 sodium hydroxid was introduced, and the mouth of the tube quickly closed with a tightly fitting rubber stopper. The tube was incubated at room temperature with a control tube.

All these tubes were examined daily for 7 days, and the growth of the yeast recorded (Table 6).

TABLE 6.—*Anaerobic growth of the pink yeast.*

Method.	Organism.	Growth.						
		24 hours.	48 hours.	72 hours.	96 hours.	120 hours.	144 hours.	168 hours.
1	Pink yeast	+	+	+	+	+	+	+
	Control	0	0	0	0	0	0	0
2	Pink yeast	+	+	+	+	++	++	++
	Control	0	0	0	0	0	0	0
3	Pink yeast	+	+	+	+	+	++	++
	Control	0	0	0	0	0	0	0
4	Pink yeast	+	+	+	+	+	++	++
	Control	0	0	0	0	0	0	0
5	Pink yeast	0	0	0	+	+	+	+
	Control	0	0	0	0	0	0	0

In the table a double plus sign (++) indicates a very abundant growth of the yeast. It is evident that the pink yeast grows readily when oxygen is excluded from the culture. The production of the pigment is in no way affected by the absence of oxygen.

RELATION TO TEMPERATURE.

As has been stated previously, the pink yeast grows best at room temperature (21° to 25° C.), whereas no growth occurs in the 37° C. incubator. The yeast grows but sparsely at refrigerator temperature (6° C.), and with but slight pigment production, the bright pink color appearing only after several days.

THERMAL DEATH POINT.

In view of these facts, it was assumed that the thermal death point of the yeast would be low, so that in determining this temperature the start was made at 36° C. The method used was taken from the Standard Methods of Water Analysis of the American Public Health Association. A 48-hour-old culture grown at room temperature was used.

TABLE 7.—*Thermal death point of the pink yeast.*

Temperature.	Result.							Temperature.	Result.						
	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.		1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.
°C.								*°C.*							
48	+	+	+	++	++	++	++	58	0	0	+	+	+	+	++
50	0	0	+	+	++	++	++	60	0	0	+	+	+	+	++
52	0	0	+	+	+	+	+	62	0	0	+	+	+	++	++
54	0	0	+	+	+	+	+	64	0	0	0	0	0	0	0
56	0	0	+	+	+	+	+	66	0	0	0	0	0	0	0

In Table 7, + indicates that the yeast was living in the tubes after exposure to the heat, and 0 indicates that there was no growth.

The temperature of 36° C., at which the experiment was started, was very much too low, and the heat had no apparent effect on the yeast up to 48° C. The temperatures below 48° C. are therefore omitted from the table, as are the records of the control tubes in which there was no growth. By raising the temperature of the bath 2 degrees at a time it has been found that the thermal death point of the yeast is 64° C. There is apparently no relation between the optimum temperature of the yeast and its thermal death point. In working on the thermal death point of the true yeast used in bread making, Wells (1917) found that the thermal death point of yeast in bread making is approximately 68° C., slightly higher than was found by the author to be the case with the pink yeast.

RELATION TO REACTION OF MEDIUM.

Experiments on the relation of the growth of the pink yeast to the acidity or alkalinity of the culture medium were conducted to determine the limits of alkalinity or acidity at which the yeast would grow; also to find the optimum reaction. Dextrose broth was prepared, and divided into 60 cc. portions. The various portions of media were titrated, and the reactions corrected, so that amounts of media were obtained with titre varying from — 9 per cent alkaline, to + 10 per cent acid.

Each portion of the different media was titrated after sterilization, and the titre recorded. The tubes were then inoculated with the pink yeast, incubated at room temperature with control tubes of the same reaction, and examined for 7 days. The growth in each tube was recorded.

TABLE 8.—*Relation of growth of the pink yeast to reaction of the medium.*

Reaction.		Growth.						
Before sterilization.	After sterilization.	24 hours.	48 hours.	72 hours.	96 hours.	120 hours.	144 hours.	168 hours.
− 1	− 0.8	+	+	+	+	++	+++	+++
Neutral.	+ 0.3	+	++	+++	+++	++++	++++	++++
+ 1	+ 1.0	+	+	++	+++	+++	++++	++++
+ 2	+ 2.0	+	+	++	++	+++	+++	++++
+ 3	+ 2.8	+	+	++	+++	+++	++++	++++
+ 4	+ 3.9	+	+	+	+	+	+	+
+ 5	+ 4.8	+	+	+	+	+	+	+
+ 6	+ 5.6	+	+	+	+	+	+	+
+ 7	+ 7.0	+	+	+	+	+	+	+
+ 8	+ 7.7	+	+	+	+	+	+	+
+ 9	+ 9.2	+	+	+	+	+	+	+
+10	+10.5	0	0	+	+	+	+	+

The terms used in describing the growth in Table 8, which are comparative only, are as follows: Very slight to slight (+); fair to very fair (++); good to very good (+++); excellent to heavy (++++). No growth appeared in any of the control tubes. Nor did growth occur in any medium the reaction of which was less than − 0.8. The growth in the acid media was very slight in the cultures which contained acid to a greater extent than 2.8 per cent. The best growth was obtained in media the reaction of which was between +0.3 and +2. In the cultures the reaction of which was +10.5 the growth was barely discernible, and this may be taken as the limit of acidity.

RESISTANCE TO DESICCATION.

Yeasts, in general, are very resistant to drying, and this has been found to be the case with the pink yeast. Pasteur (1879) studied a yeast which continued to grow after being dried for 1 year.

The pink yeast was dried on glass rods in a specially constructed drying-box. This consisted of a wooden box, the cover of which was perforated. Each one of the holes in the cover of the box was plugged with cotton, and the whole apparatus was sterilized in the autoclave at 15 pounds for 15 minutes. Corks which would fit the holes in the cover of the box were selected, and small glass rods were inserted in the corks. These were sterilized in the autoclave also. Forty-eight-hour-old cultures of the pink yeast were made in dextrose broth. The sterile rods were dipped in these cultures, and the rods, with some of the culture adhering to them, were suspended in the sterilized box. Each day one of the rods was removed from the box, and the yeast which was dried on it washed off in dextrose broth. As each rod was taken from the box the hole was plugged with a sterile cotton plug. The tubes of broth in which the dried rods were washed were incubated at room temperature, and observed for the growth of the yeast.

Up to July 1, 1918, the yeast was recovered in pure culture from rods suspended in the drying-box for 100 days. The fact that drying under atmospheric conditions apparently has no effect on the viability of the pink yeast helps to explain why the yeast, after being dried in oyster bins, etc., for several months, will readily grow when placed in a favorable environment.

RESISTANCE OF THE PINK YEAST TO DISINFECTANTS.

Early in the study of the pink yeast an attempt was made to find a disinfectant with which the infected objects in the oyster house could be washed as a method of controlling the development of the yeast. It was necessary to use a chemical which would in no way be injurious to the oysters should it come in contact with them, or harmful to the consumer in case such oysters were eaten.

Chemicals employed in the disinfection of water, such as calcium hypochlorite, or bleaching powder, and copper sulphate, were first used. Neither of these proved efficient in dilutions such as it was feasible to use. Since the yeast was known to be surrounded by a capsule, probably of a lipoid nature, soap and soapine solutions were tried, in the hope of breaking the capsule and thus killing the yeast. When this failed, copper sulphate was mixed with the soap solution in the belief that the soap might break the capsule and allow the copper sulphate to act. This treatment, however, had no effect on the viability of the yeast. A coal-tar disinfectant in a dilution of 1 to 1,000 parts was not effective, and sodium sulphite and copperas in low dilution were also found to be without effect. Formalin, which was not used at first because of its odor and the fact that molds often grow readily on it, was finally resorted to. It was found that formaldehyde gas present in 1 to 2,500 parts of water quickly kills the yeast.

In testing the effects of disinfectants on the yeast, the following method was employed: The yeast was grown in 9 cc. amounts of dextrose broth for from three to five days. The disinfectant to be tested was diluted in such a manner that 1 cc. added to the 9 cc. of culture would give the required dilution. In the case of calcium hypochlorite the amount of available chlorin was first determined, and dilutions prepared which contained known amounts of the chlorin. When testing formalin, dilutions were made from the commercial 40 per cent solution, and the amount of formaldehyde gas in the final dilution was computed. The cultures to which the disinfectant had been added were set aside for known lengths of time;

then subcultures were made on dextrose agar slants. These agar cultures were examined daily for the growth of the yeast.

After it was found that formaldehyde, 1 to 2,500, would kill the yeast in broth cultures, this disinfectant was applied to the oyster house. Early in the morning the bins and benches of the opening house were swabbed, and the pink yeasts were recovered from the swabs. The bins and benches were then scrubbed thoroughly by means of brooms and running water from a hose. Following the scrubbing the woodwork was washed down with formaldehyde solution, and the bins left unused during the day. Later in the afternoon the bins and benches were swabbed again, and no pink yeasts were obtained.

It is apparent that if this procedure is followed the principal source of contamination can be removed. It is well to leave the formaldehyde solution on the woodwork overnight or during the entire day. The same procedure may be followed in washing the tubs, wheelbarrows, and other contaminated objects in and about the oyster house.

DISCUSSION OF RESULTS.

In discussing the relation of the pink yeast to the oyster industry it will be well to consider the method of handling the oysters in preparing them for shipment, as practiced in most of the establishments in Rhode Island where this study was conducted, to note the possibilities of contamination with the pink yeast in the process, and draw some conclusions from the data already collected as to the source of the contamination.

The oysters are caught in large dredges and dumped on the deck of the boat, after which they are shoveled back along the deck to a large pile amidship. The day's catch of oysters is taken to the oyster house, and unloaded by shoveling the oysters into wooden tubs in which they are carried back into the house and dumped into bins. The oysters are opened into metal dippers and taken to the washroom to be cleansed and iced for shipment. They are first washed on a skimmer with fresh water from a hose, then put into tubs of salt water for 15 minutes, at the end of which time the salt water is replaced with fresh water, and a piece of ice added. When the oysters are thoroughly chilled they are washed on a second skimmer with salt water. The oysters, thus washed, are measured into 1-, 2-, or 5-gallon cans, and covered with a tight-fitting tin cover which is not, however, air-tight. These cans, which are cylindrical, are packed in square wooden boxes of the proper size to hold the can, leaving a space in each corner which is filled with cracked ice. The cover of the box is nailed on, and the oysters thus packed are shipped.

As stated elsewhere (page 1), oysters packed in this way and sent for long distances are often pink when they reach their destination.

The possibilities of contamination in the process of handling just described may now be considered. Disregarding, for the moment, the fact that the oysters may contain the yeasts when taken from the beds, it may be noted that they are immediately brought in contact with the deck of the boat, which is known to be infected. This, then, is one possibility of contaminating the outside shell of the oyster. Later the oysters are placed in tubs, wheelbarrows, and bins which are also known to be infected, presenting further opportunities for contamination. From the time that the oysters are taken from the water until they are opened in the oyster house there is every chance for the outside of the oyster shell to become contaminated with the pink yeast, but practically no chance for the contents of the oyster shell to be contaminated, since the oyster closes its shell and remains tightly closed during the process of handling. On opening the oysters, however. some of the dirt on the outside of the shell is unavoidably transferred to the dipper of opened oysters. Although these oysters are thoroughly washed, small organisms like the pink yeast are not easily washed off, and may be carried along into the shipping cans.

The data collected show that the house, the boats, and the utensils within and about the house are heavily infected with the pink yeast, that no yeasts were found in the mud at the bottom of the bay, and that yeasts were present in a very small number of the water samples. While it is true that some oysters when taken from the water contain small numbers of pink yeast, such oysters are not often found, and it is not probable that this is an important source of infection. The results of this investigation indicate that the source of the trouble lies in and about the oyster house. If measures are taken for preventing contamination there, the trouble from the pink yeast will be eliminated.

The fact that the pink yeast is occasionally found in water samples and on oysters taken from the oyster beds may possibly be explained in the following manner. Every season oysters are brought to the oyster house, where they are culled, and then taken back to the oyster beds. Such oysters are contaminated in the process of handling and no doubt carry back the pink yeast with them to the bay. A further explanation, which seems more probable, is that the shells that are carried down the bay and planted in the summer may be bearers of the pink yeast. After opening the oysters in the oyster house the shells are dumped in a large pile on the adjacent grounds and left there until June or July, when they are taken to the oyster beds and planted in order to give a smooth, clean surface for the oyster spat and also to furnish calcium salts for the growing oyster. These shells are contaminated with the pink yeast after passing through the house, and it is known that the pink yeast grows aboundantly in the shells and in the moisture which collects at the bottom of the pile during months in which they lie in the yard. When these shells are taken to the beds it is probable that large numbers of the pink yeasts are carried with them.

As a method of control it is recommended that all the bins, benches, tubs, etc., be scrubbed thoroughly with water and then washed with a 0.04 per cent solution of formaldehyde gas, which is equivalent to 1 part of formaldehyde to 2,500 parts of water. Such a solution may be prepared by adding 1 part of commercial formalin to 1,000 parts of water. The washing should be done in the fall, before any oysters are brought to the opening house; and bins, benches, tubs, etc., should also be washed several times during the winter whenever convenient.

SUMMARY.

1. The organism causing a pink color in shipped oysters is a yeast-like fungus.
2. This pink yeast was found in large numbers in the oyster house and on the utensils in and about the house. Of the examinations of the house and utensils 73.9 per cent were positive.
3. The yeast was found less frequently in oysters before they were brought to the oyster house. Of the oyster samples analyzed 26.3 per cent were positive.
4. Occasionally the yeast was found in deep and surface water samples from the oyster beds. Three and six-tenths per cent of the deep samples and 2.8 per cent of the surface samples were positive.
5. The yeast was not found in mud samples from the bottom of the bay.
6. The yeast is elliptical, measuring 6 by 4 micra. It reproduces by budding and does not form spores. Staining by Gram's method is peculiar.
7. The yeast grows at room temperature (21° to 25° C.), and does not grow at 37° C. The best medium for its growth is dextrose agar, although it can be grown on gelatin, potato and blood serum, and in broth and milk.
8. The yeast does not produce acid, gas, nor alcohol in carbohydrate media. It inverts saccharose. It does not produce indole nor phenol. It reduces nitrates to nitrites and ammonia.
9. The yeast produces a pink pigment which is insoluble in water, slightly soluble in alcohol and carbon disulphid, somewhat more soluble in chloroform, and very soluble in ether.
10. The yeast grows abundantly in the absence of oxygen. Its thermal death point is 64° C. The limits of alkalinity and acidity for its growth are -0.8 and $+10.5$. It grows best between $+0.3$ and $+2.0$. It resists drying for at least 100 days.

11. Formaldehyde gas, 1 part to 2,500 parts of water (0.04 per cent solution), kills the yeast.

12. The pink yeast belongs to that group of yeast-like forms called Torulæ. Since the complete life cycle of the Torulæ is unknown they are placed among the *Fungi Imperfecti.*

13. The principal source of contamination of oysters with the pink yeast is in the process of handling in the oyster house.

14. The shell pile of the opening house is a favorable place for the yeast to grow, and when the shells are later deposited on the oyster beds they serve to distribute the yeasts in the water.

15. For the purpose of preventing the infection of opened oysters by the yeast, the house and utensils should be washed occasionally through the opening season with a 1 to 2,500 formaldehyde solution (formalin, 1 to 1,000).

BIBLIOGRAPHY.

1850. Fresenius. Beitr. Mykol., Frankfort, 1850–63.
1872. Schröter and Cohn. Beitr. Biol. Pflanz., **1**: 110.
1879. Pasteur. Etudes sur la bière.
1886. Elfving. Oversigt Finska Vet. Soc. Forhandl., 28.
1887. Lindner. Wochschr. Brau., **4**: 853.
1889. Laurent. Centr. Bakt., **5**: 794.
1890. Demme. Centr. Bakt., **9**: 271.
1890. Kayser. Le Cidre, **3**: 371.
1890. Van der Hulle and van Laer. Abst. in Wochschr. Brau., **8**: 984.
1891. Kramer. Abst. in Chem. Centr., **2**: 707.
1892. Krueger. Molkereizeituug Hildesheim, nos. 34–38.
1892. Ward. Abst. in Wochschr. Brau., **19**: 75.
1894. Fischer and Brebeck. Zur Morphologie, Biologie u. Systematik d. Kahmpilze d. Monilia candida Hansen u. d. Soorpilze. Jena.
1895. Peglion. Staz. sper. agrar. ital., **28**: 369.
1896. Bay. Centr. Bakt., **2**: 259.
1896. Swan. Centr. Bakt., **2**: 1.
1898. Hansen. Centr. Bakt., **4**: 862.
1899. Meissner. Centr. Bakt., **5**: 232.
1900. Reinmann. Centr. Bakt., **6**: 131.
1902. Hansen. Compt. rend. trav. lab. Carlsberg, **5**: 68.
1903. Hartmann. Centr. Bakt., **10**: 463.
1903. Herzog. Z. physiol. Chem., **38**: 396.
1903. Janssens and Mertens. La Cellule, **20**: 351.
1912. Klein and Gordan. Centr. Bakt., **35**: 601.
1914. Bates and Round. The Occurrence of Pink Color in Shucked Oysters Noted During the Fall of 1914. U. S. Dept. Agr., Bur. Chem. Prelim. Rept. (unpublished).
1914. Henrici. J. Med. Res., **30**: 409.
1916. Chapman. Biochem. J., **10**: 548. Bull. Inst. Pasteur, **15**: 616.
1917. Wells. Vermont Exp. Sta. Bull. 203.

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